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Contractile properties of knee-extensors in one single family with nemaline myopathy: central and peripheral aspects of muscle activation

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Summary

Patients with nemaline myopathy, a muscle disorder primarily affecting the thin filaments, suffer from weakness which is poorly understood. As disturbed excitation-contraction coupling has been suggested as a possible mechanism, the present study was designed to investigate whether the contractile properties of the knee-extensor muscles in patients from a single family with nemaline myopathy were different from able-bodied individuals. To assess central neural as well as more peripheral intrinsic aspects of muscle activation, isometric voluntary and electrically elicited quadriceps contractions were evoked at different knee angles. Interestingly, across the range of 30–70° of knee flexion, the capacity to achieve maximal voluntary activation of the muscles, assessed by a super-imposed stimulation technique, was significantly higher in patients compared with controls. Furthermore, the torque–frequency relation differed between groups, with the muscles of patients producing higher torques at low (twitch and 10 Hz) stimulation frequencies relative to maximal (150 Hz) stimulation than controls at both 30° and 60° of knee flexion. These results suggest that no impairment was present at relatively low activation frequencies. It may, however, be indicative for a reduced cross-bridge attachment as part of the excitation–contraction coupling specifically at high activation frequencies. In conclusion, the quadriceps weakness observed in this specific patient group cannot be explained by an impaired capacity to maximally activate these muscles. However, the data of relatively high torques produced at submaximal activation frequencies are compatible with the hypothesis that patients with nemaline myopathy may have an impaired acto-myosin interaction specifically at high levels of activation.

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Introduction

Patients with nemaline myopathy, a congenital muscle disorder, suffer from a mild to severe weakness, predominantly of proximal limb muscles but also of facial, respiratory or distal muscles. Until now, the mechanisms responsible for this strength loss are unclear. For instance, the presence of nemaline bodies (rods) typically seen in muscles of patients seems not directly related to the degree of weakness (Wallgren-Pettersson *et al.*, 1988; Shimomura & Nonaka, 1989). Because loss of strength may significantly constrain their daily life activities (Sanoudou & Beggs, 2001) it is important to obtain information about the nature of the weakness and the circumstances under which it occurs. This information may help to optimize treatment and rehabilitation programmes for reducing these functional limitations.

To date, five genes have been identified to be associated with nemaline myopathies and all five of these genes have been shown to encode for components of thin filaments (Wallgren-Pettersson & Laing, 2001). In a recent study, we described a family with a phenotype that was not linked to one of these known genes (Gommans *et al.*, 2003). The histological features of the muscle biopsies from these patients, such as presence of nemaline rods in the absence of other conditions sometimes associated with rods (Gommans *et al.*, 2002) showed great similarities with those from the known gene mutations (Wallgren-Pettersson & Laing, 1996). Based on this information, it is assumed that this disease also involves the thin filaments, possibly affecting proteins

involved in excitation–contraction coupling which would prevent, for instance, adequate acto-myosin interaction. Interestingly, in this particular family, weakness of the knee-extensor muscles was more pronounced with the knee joints more flexed (i.e. longer muscle length) when compared with able-bodied control individuals (Gerrits *et al.*, 2003). Furthermore, the optimum position for torque production was obtained at lower knee-flexion compared with controls, indicating adaptations in the force–length relation of the muscles involved. However, altered force–length characteristics could not fully explain the overall weakness observed in these patients.

Other potential mechanisms for this impaired force generation may relate to altered processes of excitation–contraction coupling, defined as the sequence of events leading from the action potential initiated at the neuromuscular junctions to cross-bridge attachment. When the *relative* torque–angle relation of our patients was considered, we observed muscle weakness at maximal activation especially at shorter than optimum length (Gerrits *et al.*, 2003) and this confirmed the results we obtained from a mouse model for nemaline myopathy (De Haan *et al.*, 2002). These results may be indicative for poor activation specifically at short sarcomere length, which may be even more pronounced at submaximal muscle contractions. To date, there is no information about activation properties and effects of muscle length in patients with nemaline myopathy. Furthermore, from a clinical perspective it seems relevant to investigate submaximal muscle contractions as these are more frequently used during daily-life activities than maximal contractions. Peripheral aspects of contractile function, such as the influence of activation frequency irrespective of voluntary command, can be reliably investigated with electrical stimulation (Gerrits *et al.*, 2001) and this methodology has been used to assess muscle function in various patients groups (Gerrits *et al.*, 1999; De Haan *et al.*, 2000; Scott *et al.*, 2006). The purpose of the present investigation was to assess the isometric contractile properties of the knee-extensor muscles during electrically evoked contractions at different knee-flexion angles in a five-generation family with nemaline myopathy (Gommans *et al.*, 2002) and to compare results with those of able-bodied individuals. In addition, although it seems most likely that the nature of the muscle weakness observed in nemaline myopathies is primarily associated with disturbed processes within the muscle fibres, altered neural drive to the muscles (i.e. central aspects of muscle activation) during maximal voluntary efforts cannot be excluded. Therefore, we additionally assessed the capacity to achieve maximal voluntary activation by means of a modified superimposed stimulation technique. This study extends on the data obtained from the same subjects and published recently (Gerrits *et al.*, 2003).

Methods

Subjects

A total number of 19 subjects volunteered for this study. Ten subjects were patients (four males; age, 44 ± 4 years) and all members of the five-generation family with a particular phenotype of autosomal dominant nemaline myopathy (Gommans *et al.*, 2002). All patients complained of mild to moderate muscle weakness in the proximal limbs (difficulties with climbing stairs, jumping, running and lifting heavy objects) which started in early childhood. Besides weakness patients complained also of slowness in movement. Physical examination showed muscle weakness in the neck flexors and proximal limb muscles [MRC (Medical Research Counsil) grade 4]. The complaint of slowness could not be detected on regular neurological examination or electromyographic examination, but was confirmed physiologically (Pauw-Gommans *et al.*, 2006) by showing lower contractile speed in the quadriceps muscle.

Nine healthy subjects (three males; age, 49 ± 9 years), including four members of the nemaline myopathy family, served as controls. None of the subjects had cardiovascular, neurological or musculoskeletal problems. Subjects signed written informed consent after they had received careful explanation about testing procedures and involved risks. The local medical ethical committee approved the study.

Procedure

Subjects performed a series of voluntary and electrically elicited quadriceps contractions. All subjects were asked to refrain from strenuous exercise for 48 h prior to the experiments. Before experiments started, subjects were familiarized with the test procedures, such as electrical stimulation. Furthermore, subjects were trained to perform a maximal voluntary contraction of approximately 3-s duration. Isometric knee-extension torque was measured with the subjects seated on a custom-built computer-controlled lower limb dynamometer. The lower leg was connected to the lever arm of the dynamometer with the hip flexed at approximately 60° and the knee flexed at different angles between 30° and 70° flexion (0° corresponds with full extension).

Padded straps around the pelvis and upper body minimized undesired movements of the hip during the measurements. Furthermore, care was taken that the axis of the lever arm was always aligned with the axis of the knee joint (lateral femur condyl). Knee-extension torques (0·001-nm resolution) were measured at the motor axis and are therefore independent of the length of the lever arm. Torque signals were digitized (1000 Hz) and stored on disc for immediate and offline analysis.

Electrically evoked contractions

A constant current electrical stimulator (model DS7A; Digitimer Ltd, Hertfordshire, UK) delivered square-wave electrical pulses (0·2-ms duration) to the quadriceps muscle via two self-adhesive surface electrodes (8 × 13 cm; Schwa-Medico B.V., Nieuw Leusden, the Netherlands). Electrodes were placed over the medial distal part and the lateral proximal part of the quadriceps muscle. The computer controlled the number and frequency of the electrical pulses delivered by the stimulator.

Electrically evoked contractions were obtained from the left quadriceps at 30° and 60° knee flexion. To activate a representative part of the muscle without provoking substantial discomfort for the subjects the stimulation current was set at a submaximal intensity. This current intensity was equal to a level that produced approximately 50% of the maximal voluntary torque at 150 Hz. This procedure was repeated for each knee angle. The intensity was then maintained at all other stimulation frequencies. Subsequently, a randomized series of twitch and tetanic contractions (700 ms) at 10, 20, 50 and 150 Hz was evoked, each contraction separated by 2-min rest. Apart from the torque responses at each of these frequencies, we obtained the half relaxation time (HRT) from the 150-Hz signal. HRT was defined as the time taken for torque to decline from 50% to 25% of the peak torque. Additional contractile characteristics are described elsewhere (Pauw-Gommans *et al.*, 2006). To minimize possible variations in muscle temperature which would affect the measurements, room temperature was kept constant at ~22°C and subjects were in the room at least 45 min before the start of the testing.

Voluntary contractions

Subjects were asked to perform two maximal voluntary knee extensions at five different knee-flexion angles (30°, 40°, 50°, 60° and 70°), which were randomly assigned to the right limb and separated by 2-min rest. The highest torque produced during these attempts was defined as maximal voluntary torque. During each voluntary effort, subjects received substantial verbal encouragement and visual feed-back to achieve maximal performance. The torque data are presented elsewhere (Gerrits *et al.*, 2003). However, some of the results (i.e. patient torques relative to control data) are shown in the present report as well. A modified super-imposed stimulation technique was used to estimate the degree of voluntary activation during each attempt. For this purpose, a brief train of three pulses at 300 Hz (triplet) was imposed on the relaxed quadriceps muscle (Kooistra *et al.*, 2005). These high frequency stimulations produce maximal responses in terms of torque production as well as the rate of torque production (De Haan, 1998), thereby limiting the sensitivity to, for instance, length-dependant changes in calcium sensitivity and improving signal-to-noise ratio. Subsequently, the same triplet was imposed on top of the plateau phase of the maximal voluntary contraction. The stimulation current for the triplets was set at supra-maximal intensity to improve the signal–noise ratio. All subjects could tolerate these stimulations, most likely due to the short duration of the stimulation. The degree of voluntary activation during a maximal voluntary contraction was estimated calculating the 'voluntary activation index' with the equation:

$$\text{Voluntary activation index (\%)} = \left(1 - \frac{\text{Superimposed triplet troque}}{\text{control triplet torque}}\right) \times 100\%,$$

where the superimposed triplet torque is the extra torque produced by the triplet on top of the voluntary contraction and the control triplet torque is the torque produced by the same triplet imposed on the relaxed muscle (Fig. 1).

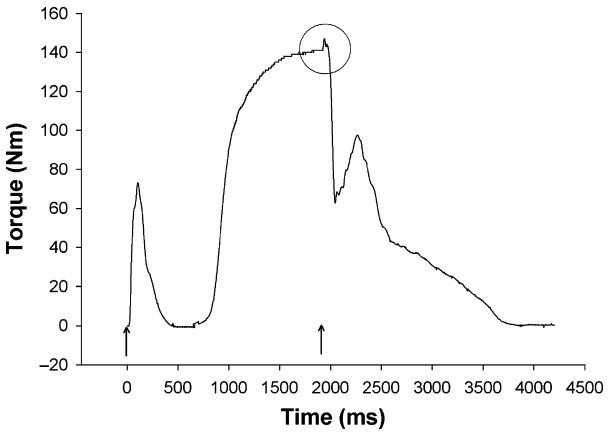

Figure 1 Typical example of a maximal voluntary contraction with superimposed stimulation. Stimulation was applied at rest and on top of the voluntary contraction (indicated by arrows).

Data analysis and statistics

Offline analysis of torque recordings was performed with applications using custom MATLAB software packages (The Mathworks Inc., MA, USA). To examine differences in maximal voluntary torque, degree of voluntary activation and contractile speed at different joint angles between patients and control subjects, repeated-measures factorial analyses of variance (ANOVA) with 'angle'(five levels) as within subjects factor and 'group'(two levels) as between subjects factor were performed. Furthermore, a repeated-measures factorial ANOVA with 'stimulation frequency' (five levels) and 'angle' (five levels) as within subject factors and 'group' (two levels) as between subjects factor was used to determine if there were any differences between groups with stimulation frequency, joint angle, and their interaction on torque production. *Post hoc* simple contrast analysis was used to study differences between repeated measures and *post hoc* Bonferroni analyses were performed to examine differences between groups, where appropriate. All data are presented as mean ± SEM unless otherwise indicated, and levels of significance were set at $P<0{\cdot}05$.

Results

One of the control subjects (female member of the nemaline myopathy family, 70 years) could not tolerate the electrical stimulation and produced unreliable torque signals. Data from this subject were therefore discarded from the final analyses leaving a total number of eight control subjects.

Maximal voluntary activation and torque

For the range of joint angles (30–70°) studied (Fig. 2) the patients achieved a higher voluntary activation index (ranging

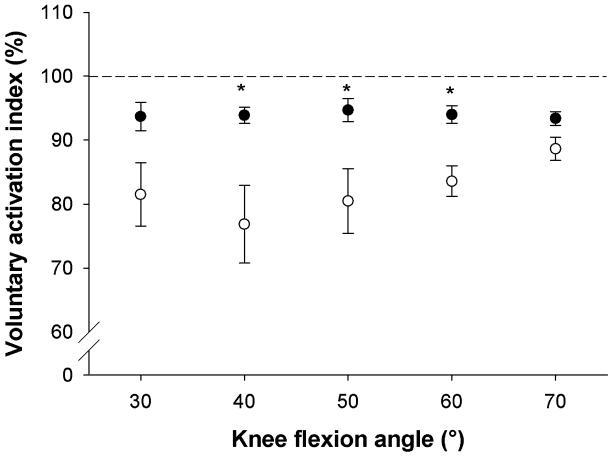

Figure 2 Voluntary activation index versus knee-flexion angle of quadriceps muscle in patients with nemaline myopathy (●) and control subjects (○). Error bars reflect SEM. *Indicates significant difference between groups, P<0·01.

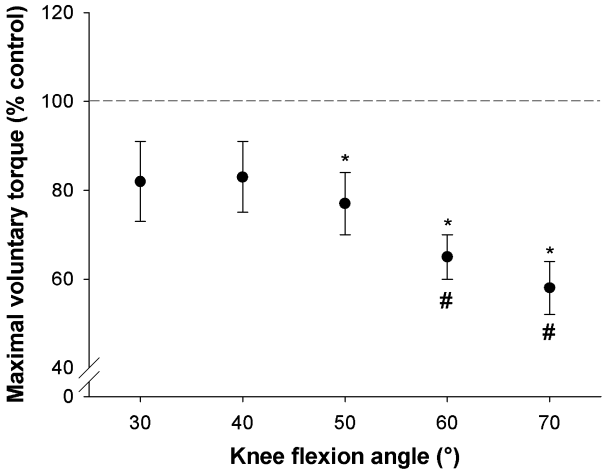

Figure 3 Normalized maximal voluntary torque of quadriceps muscle at different knee- flexion angles of patients with nemaline myopathy expressed as a percentage of mean control data. Error bars reflect SEM. *Indicates significant difference between groups (P<0·01); # indicates significantly different from preceding joint angle (P<0·01).

between 93 ± 1 and 95 ± 2 across angles) compared with control subjects (ranging between 77 ± 6% and 89 ± 2% across angles), indicated by a significant main effect of group (P<0·01). Voluntary activation index varied with knee-flexion angle and this was different between patients and controls (group × angle, P<0·05). In controls, voluntary activation index tended to decrease with lower knee-flexion angle (P = 0·06), whereas no angle dependency could be observed in the patients (P = 0·948). *Post hoc* analysis showed that group differences were present at 40°, 50° and 60° (P<0·01).

Results of maximal voluntary torque production at the different knee-flexion angles are presented elsewhere (Gerrits *et al.*, 2003). Briefly, patients produced lower maximal voluntary torques compared with controls, indicated by a significant main effect of group (P<0·05) and torque–angle relations also differed between groups (torque × angle, P<0·05). *Post hoc* analysis revealed that patient muscles were weaker than controls at 50° (P<0·05), 60° and 70° (P<0·01) (Fig. 3). In addition, this weakness increased with greater knee-flexion as the torques produced by the patients relative to average control values at that angle reduced from 77 ± 7% at 50° to 65 ± 5% at 60° (P<0·01), and to 58 ± 6% at 70° (P<0·05).

Torque–frequency relation and contractile speed

The torque responses to a series of different stimulation frequencies at two different joint angles (60° and 30° knee-flexion angle) are depicted in Fig. 4.

With respect to absolute torque responses (Fig. 4a,b), there was a significant difference in the frequency response between patients and controls, irrespective of joint angle (frequency × group, P<0·001). At 60°, knee-flexion patients showed lower torques than controls at 150 Hz and 50 Hz (P<0·01), which was the results of setting the stimulation intensity at the same relative intensity in both patients and control subjects (i.e. ∼50% of maximal voluntary torque). It is notable, however, that at the lower stimulation frequencies (i.e. twitch, 10 Hz and 20 Hz) differences between groups were absent. Furthermore, although at 30°, the difference between groups in terms of frequency response was also observed (P<0·01), *post hoc* analysis revealed no group differences at each individual frequency tested.

To obtain information about the influence of activation frequency irrespective of muscle strength torque responses are expressed relative to 150-Hz stimulation (Fig. 4c,d). Interaction effects between frequency, angle and group (P<0·01) indicated differences in the torque–frequency relation between groups, which was different for the angles studied. There was a significant angle effect on the frequency response (angle × frequency, P<0·01) reflected by a downward shift at 30° compared with 60° in both patients (P<0·001) and controls (P = 0·001). *Post hoc* analysis showed a significant decline in twitch, 10 Hz, 20 Hz torque response in the patients (P<0·01) as well as in the controls (P<0·01). In addition, differences in the torque–frequency relation between patients and controls were observed both at 60° (P<0·001) and 30° (P<0·05) At 60° knee-flexion, patients produced higher relative torques at low stimulation frequencies (twitch and 10 Hz, P<0·01) compared with control subjects (Fig. 4c). At 30° knee-flexion, patients still produced higher relative torques (10 Hz, P<0·01) despite a greater downward shift in the relation from 60° to 30° compared with controls (Fig. 4d, P<0·05).

Patients and controls differed significantly with respect to the contractile speed of their knee-extensors, indicated by a significant main effect of group (P<0·01). HRT was significantly longer in the patients compared with controls at 60° [59 ± 7 versus 32 ± 2 ms, (P<0·01)] as well as at 30° [39 ± 3 versus 27 ± 2 ms, (P<0·01)] knee-flexion. Furthermore, a significant interaction effect between angle and group (P<0·05) indicated that the ∼34% decline in HRT from 60° to 30° in the patients was greater than the ∼16% decline in controls.

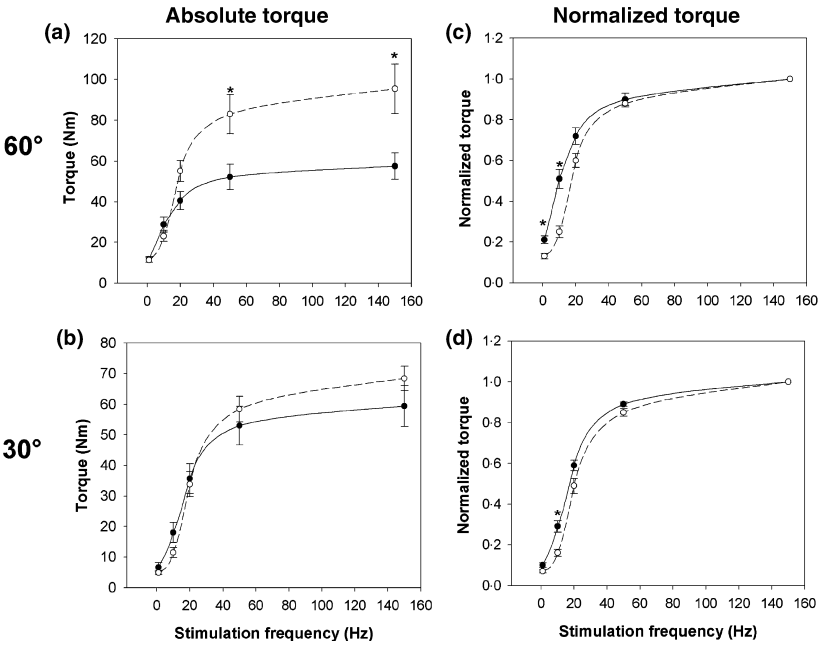

Figure 4 Torque–frequency relationship of quadriceps muscle in patients with nemaline myopathy (●) and healthy control subjects (○) at 60° (a, c) and 30° (b, d) knee-flexion angle. Panel (a) and (b) show the absolute torque data. Normalized torque data (relative to 150-Hz torque) are depicted in panel (c) and (d). Error bars reflect SEM. *Indicates significant difference between groups, $P<0{\cdot}01$.

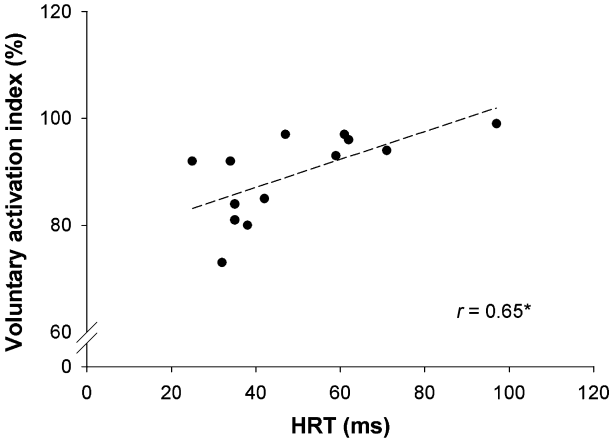

Figure 5 Relationship between half relaxation time (HRT) and voluntary activation index at 60° knee-flexion including data of patients and control subjects. *Indicates a significant correlation ($P<0{\cdot}05$).

Pearson correlation coefficients were calculated to determine whether the difference voluntary activation between groups was related to differences in contractile speed. A significant correlation was observed between HRT and voluntary activation index at 60° knee-flexion ($r = 0{\cdot}65$, $P<0{\cdot}05$) by including both patient and control data (Fig. 5).

Discussion

The most important finding of the present study was that patients from a single family with a particular phenotype of nemaline myopathy (Gommans *et al.*, 2002) exhibited different contractile function of their knee-extensor muscles compared with able-bodied controls. This different muscle performance was associated with alterations in 'central' as well as more intrinsic or 'peripheral' aspects of muscle activation. The voluntary activation index obtained during maximal voluntary contractions was higher compared with controls at nearly all angles tested. Furthermore, during electrically elicited contractions isometric torque at submaximal activation was relatively higher in the patients compared with controls both at high and low knee-flexion angles.

Central aspects of muscle activation: maximal voluntary torque

Recently, we demonstrated weakness of the knee-extensor muscles of patients with a novel phenotype of nemaline myopathy, especially at high knee-flexion angles (Gerrits *et al.*, 2003). One of the purposes of this study was to investigate whether this weakness was accompanied by a change in the capacity to maximally activate the muscles during voluntary contractions.

We found no evidence for an impaired voluntary activation as the patients even achieved a higher voluntary activation index at nearly all knee-flexion angles tested. In addition, in controls voluntary activation was related to the knee-flexion angle with lower activation at 40–50° knee-flexion angles. Previous studies have described a similar joint angle dependency for muscle activation (Suter & Herzog, 1997; Becker & Awiszus, 2001) and authors suggested that this phenomenon might result from muscle inhibition due to high strains on structures of the knee-joint in these specific joint angles. In contrast, no angle dependency was observed in the patients. It seems that this different response cannot be explained by differences in muscle strain between groups because voluntary torque production of patients is lowest (58% of control) at high knee-flexion (70°), where no group differences in activation are present and this

increases to 80% at 40° where differences in activation are most pronounced.

The absence of a reduced voluntary activation capacity seems to fit well with the nature of the disease, which is primarily related to the sarcomere structure of the muscle (Wallgren-Pettersson & Laing, 2001). The *higher* activation levels observed are, however, a remarkable and rather surprising observation of the present study.

A possible explanation for the higher activation in patients with nemaline myopathy may be related to differing muscle fibre compositions between the patient and control groups. Muscle biopsies taken from four of the patients demonstrated a type I muscle fibre predominance, which is a common feature of nemaline myopathies (North *et al.*, 1997). Two of the nemaline patients showed relatively high (>65%) and two even extremely high (>80%) percentages of type I fibres, whereas two of their relatives serving as controls had normal fibres type composition (∼40% type I) when compared with previous reports (Simoneau *et al.*, 1985). The fibre type composition of a given muscle determines its contractile characteristics, with faster properties in a muscle comprising higher proportions type II fibres (Harridge *et al.*, 1996; Gerrits *et al.*, 1999). Accordingly, we observed slower contractile properties in the knee-extensors of our subjects with a relatively high fraction of type I fibres (Pauw-Gommans *et al.*, 2006). Moreover, it has been shown in animals (Close, 1967; Kernell *et al.*, 1983) and in humans (Harridge *et al.*, 1996) that the contractile speed of muscle (fibres) dictates the stimulation frequency necessary to generate tetanic tension, with lower stimulation frequencies necessary to obtain the same level of relative force production compared to fast muscles. Based on this, it may be suggested that lower discharge rates are required to maximally recruit a relatively 'slow' muscle, which may be easier to achieve. In fact, the HRTs of our subjects, used as a measure of contractile speed, showed to be significantly related to the voluntary activation index. These results support the suggestion that the altered contractile characteristics, most likely due to high proportions of type I muscle fibres, may (partly) account for the higher voluntary activation capacity observed in the patients.

A second plausible explanation for the observed higher voluntary activation may be of a more adaptive nature. Patients with muscle weakness with a peripheral origin may simply compensate for this weakness by increasing the central drive to the muscles.

Since the work of Henneman *et al.* (1974) it is long known that the muscle force required during certain motor tasks can be modulated by increasing the number of motor units recruited or by increasing the firing frequency. Because the muscles of the patients are weak, during everyday activities that require only moderate muscle forces these muscles would already need to work at higher activation levels than would normally be the case in healthy individuals. As a result, the patients would have to use maximal activation levels more frequently than healthy individuals. Skill acquisition can alter motor unit recruitment strategies (Bernardi *et al.*, 1996) and from our own experience (Kooistra *et al.*, 2005) we know that most healthy individuals can reach voluntary activation levels of >95% of their quadriceps muscles when maximal voluntary contractions are practiced frequently. Consequently, although both patient and control groups were relatively inexperienced with respect to the knee-extensor measurements it may be speculated that the patients were more skilled in achieving maximal activation at the time of the testing procedures thereby reaching higher voluntary activation levels than the controls. Such mechanism may also apply to various other (primary) muscle disorders.

Peripheral aspects of muscle activation: frequency response

In a previous study, using the same subjects as in the present report, we presented a reduction in the maximal torque production specifically at shorter than optimum muscle lengths (Gerrits *et al.*, 2003), which agreed with observations in transgenic mice with a mutation in the *TPM3* gene (De Haan *et al.*, 2002). Based on studies on isolated cell models for nemaline myopathy, reporting reduced actin affinity (Moraczewska *et al.*, 2000) or calcium sensitivity (Michele *et al.*, 1999), we suggested that impaired excitation–contraction coupling may be responsible for the observed force deprivation specifically at short sarcomere length in our subjects. In addition, if disturbed calcium handling would indeed underlie the impaired force generation at maximal activation, an impaired contractile function at submaximal muscle contractions may also be expected. However, we found no reduced torque production at low stimulation frequencies in the knee-extensor muscles of the patients. Hence, we have no evidence of impaired activation via these processes, which is in agreement with the results on transgenic mice (De Haan *et al.*, 2002).

An interesting observation of the present study was that the low frequency range of the patients shifted even *upward* when compared with controls, both at high and low muscle length. For instance, at 60° knee-flexion twitches were ∼60% higher and 10 Hz torques were even twice as high compared with control values. It is noteworthy that at this angle the knee extensors of the patients were activated at a relatively longer muscle length because the optimum position for maximal torque production was shifted towards lower knee flexion (Gerrits *et al.*, 2003) compared with controls. The force–frequency relationship shifts up with longer muscle lengths (Roszek *et al.*, 1994; De Haan *et al.*, 2003), which most likely reflect the length dependency of calcium sensitivity in striated muscle (Stephenson & Williams, 1982). We confirmed this angle dependency of the torque–frequency relationship (Fig. 4c,d) leading us to suggest that the different frequency response between groups was at least partly related to differences in the relative muscle length at which the relationship was studied. Unfortunately, for practical reasons, we were not able to study the frequency responses exactly at optimum length for each individual. However, when considering the shift in the curves from 60° to 30° knee-flexion angles for both

groups, it is likely that the difference between groups at optimum angle would not completely disappear but would only be somewhat reduced.

It was shown before in humans that relatively 'slow' muscles exhibit higher torques at low relative to high stimulation frequencies (Harridge *et al.*, 1996), Therefore, it is presumable that the differences in frequency response between patients and controls (partly) relate to a difference in fibre type composition between groups. Indeed, relatively high torques were found at 10- and 20-Hz stimulation in the four patients with a high (>65%) percentage type I fibres However, the high twitch torques cannot be explained by slow fibre characteristics.

Finally, it should be taken into regard that a relative torque–frequency relation does not exactly clarify whether differences in the relation result from an increased activation at low stimulation frequencies or from a decreased activation at high stimulation frequencies. Usually, the torque–frequency relation is interpreted expressing the low stimulation frequencies relative to the high frequencies (Harridge *et al.*, 1996; Binder-MacLeod *et al.*, 1998; Gerrits *et al.*, 1999).

This would imply that any differences in the frequency responses of muscles are associated with altered activation at submaximal stimulation. However, in the present patient group, it may be more likely that force generation at the level of acto-myosin filaments is impaired at high activation rather than that it is increased at low activation levels. The muscle biopsies taken from our patients showed a loss of myofibrilar organization (Gommans *et al.*, 2002) and it may be speculated that these ultrastructural changes lead to alterations in the proportion of available binding sites relative to potential binding sites. For instance, as the muscle fibres shorten with higher muscle torques (due to higher activation levels) the ultrastructure may change such that existing acto-myosin interactions may prevent further cross-bridge attachments. This would limit the number of available actin binding sites, thereby attenuating force production, especially at high activation and not (or less) at lower activation levels. Although speculative, such mechanism may also lead to an overestimation of voluntary activation (i.e. superimposed stimulation at high frequency may be less effective) and potentially explain why our patients, similar as transgenic mice, produced less torque at maximal activation, especially at shorter than optimum length (De Haan *et al.*, 2002; Gerrits *et al.*, 2003). Based on this reasoning it may be argued that, possibly, the high voluntary activation levels observed during maximal voluntary contractions in the patients are contaminated by a limited number of actin binding sites available at high activation.

During standard clinical evaluation of patients muscle strength is usually assessed with manual tests, which makes it difficult to obtain systematic, objective, and reliable information about the degree of muscle weakness. The results of the force–frequency relation may indicate that muscle weakness is prominent at high muscle activation but seems much less important during more regular daily-life activities, where individual motor units usually fire at frequencies between 5 and 30 Hz (Carpentier *et al.*, 2001; De Ruiter *et al.*, 2004). In this light, it is noteworthy that the patients show relatively normal muscle function during standing and walking, but typically have problems during rising from a chair or climbing stairs. Although this impaired muscle function may be related to specific weakness at relatively long muscle length (Gerrits *et al.*, 2003), this may also relate to weakness especially at higher activation levels.

It can be concluded that the knee-extensor muscles of patients of one particular family with nemaline myopathy exhibit altered contractile properties. The capacity to maximally voluntary activate the muscles was higher in patients than in controls indicating that weakness observed in the patients is not related to any central aspects of neuromuscular control. The torque–frequency relations of the muscles in the patients are shifted upwards with higher relative torque responses at low frequencies. Based on the results of this study, alterations in both intrinsic as well as central aspects of muscle function in these patients with nemaline myopathy seem primarily related to adaptations in muscle structure and fibre composition. This leads to the hypothesis that potential mechanisms for the observed weakness, and possibly also for the altered frequency response, relate to limited acto-myosin interaction specifically at high activation levels.

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